RESEARCH ARTICLE

Determination of Phenylephrine Hydrochloride and Amoxicillin in a Binary Mixture using Derivative Spectrophotometry Methods

Aseel M Aljeboree[1], Abbas Noor Alshirifi[2]

[1]*College of science for women-Chemistry Department/Babylon university-Iraq*[1]
[2]*College of Science -Chemistry Department/Babylon university-Iraq*

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ABSTRACT

A simple, precise, and economical procedure for the simultaneous estimation of phenylephrine hydrochloride (PHE) and amoxicillin (AMX) in the formulation has been developed. The absorbance values of first derivative spectrum 228,258 nm and 241 nm and second derivative spectrum 238, 277 nm and 226 nm was used for the estimation of PHE and AMX, respectively, without mutual interference. This method obeyed beer's law in the concentration range mixing of 2-150mgL^{-1} PHE with 0,20,100 mgL^{-1} AMX and 2-240 mgL^{-1} AMX with 0,20,100 mgL^{-1} PHE and the second derivative depends on the first derivative of the ratios spectra. The proposed methods are extensively validated. All the described methods can be readily utilized for analysis of pharmaceutical formulations.

Keywords: Amoxicillin, Derivative Spectrophotometry method, Phenylephrine hydrochloride, Simultaneous determination, Spectrophotometry.

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INTRODUCTION

Pharmaceuticals or drugs are extensively consumed to improve the health statuses of humans and animals,1-3 common drugs are antibiotics, analgesics, anti-inflammatory agents, lipid regulators, hormones, and β-blockers,4,5These biologically active chemicals appear in effluents of hospitals, drug factories, and landfills.6,7 Drugs have been categorized as hazardous pollutants because of their continuous-release, stability, and negative effect on the environment [8-10].

Amoxicillin (AMX),6-(p-hydroxy-α-amino phenyl acetamido) penicillanic acid, is the only phenolic penicillin, and it is used as an antibacterial drug.11 AM is one of the top-priority human and veterinary pharmaceuticals.12 Amoxicillin is widely used in veterinary practice for the treatment of gastrointestinal and systemic infections. Amoxicillin and ampicillin are known penicillins which is added to medicated feeds at the level of 250–500 mg kg−1, because of its resistance to gastric juice.

Phenylephrine hydrochloride (sympathomimetic drug) is a pharmacological agent usually used in the practice of ophthalmology to reinforce the mydriatic effect of other cycloplegic agents such as tropicamide,13-15 and used for nasal congestion, sinusitis, and rhinitis. It also acts as a mydriatic in ophthalmic solutions relieving eye redness caused by colds, hay fever, wind, dust, sun, smog, smoke or contact lenses. It can also be administered along with local anesthetics to prolong the effect of anesthesia.8,16 It is incorporated in a number of pharmaceutical preparations either alone or more frequently associated with other active ingredients. Dosage forms include tablets, syrups, eye drops, and injections.

For the simultaneous determination of two or more active compounds in the same mixtures without a separation step, several spectrophotometric methods, such as classical derivative spectrophotometry, has been utilized. Derivative spectra could be obtained by optical, electronic, or mathematical methods. Optical and electronic techniques were used on early UV-Vis spectrophotometers but have largely been superseded by mathematical techniques.17

Derivative spectrophotometry is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed of unresolved bands, and for eliminating the effect of baseline shifts and baseline tilts. It consists of calculating and plotting one of the mathematical derivatives of a spectral curve; this feature leads to narrowing bands and as a consequence to separate the overlapped peaks. Derivative spectrophotometry is now a reasonably prized standard feature of modern micro-computerized UV spectrophotometry.18

**Author for Correspondence:annenayad@gmail.com*

EXPERIMENTAL

Instrumentation

All absorption spectra were recorded by U.V.-Visible double beam 1800 Shimadzu (Kyoto-Japan) spectrophotometer on a range of 200-300 nm. The resulted absorption data were digitalized, plotted, and manipulated by Shimadzu 1800 software (UV Prob 2.34) to obtain the first and second.

Preparation of standard solutions and calibration

Stock standard solutions containing 1g phenylephrine hydrochloride or amoxicillin were dissolved in 1000ml distilled water. Standard solutions of both drugs were prepared individually by dilution of the stock solutions with distilled water for spectrophotometric methods to obtain concentration range of 2-150 mg/L for phenylephrine hydrochloride and 2-240 mg/L for Amoxicillin respectively.

For derivative spectrophotometric method (D1)

The values of the D1 amplitudes were measured at 241 nm(zero-crossing of phenylephrine hydrochloride) and 228, 258 nm(zero-crossing of Amoxicillin) for the determination of Amoxicillin and phenylephrine hydrochloride, respectively.

Calibration Curves for phenylephrine HCl

Under the mentioned optimum conditions for D1 and D2 modes in selected ranges of wavelengths, Calibration curves were obtained for the assay of phenylephrine HCl via UV-spectrophotometric method. The same ideas used previously to utilize derivative modes for the quantitative analysis of phenylephrine HCl are used zero-cross measurements

The recorded spectra using D1 mode for a set of solutions containing (2-150 mg. L^{-1}) PHE in the presence of different concentrations of AMX (0, 20, 100 mg. L^{-1}) are shown in

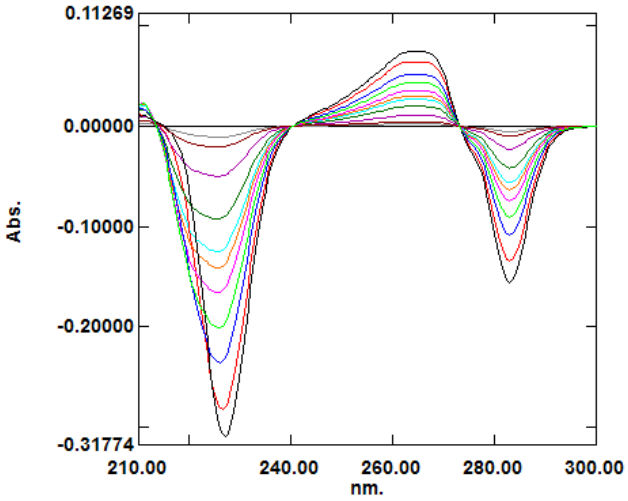

Figure 1: First derivative spectra of (5-150 mg. L-1) phenylephrine HCl concentration.

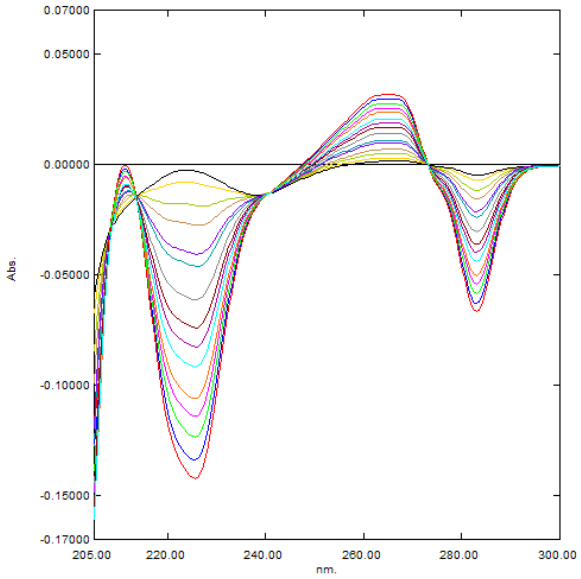

Figure 2: First derivative spectra of mixture contain (5-150 mg. L^{-1}) phenylephrine HCl in the presence of (20 mg. L^{-1}) amoxicillin

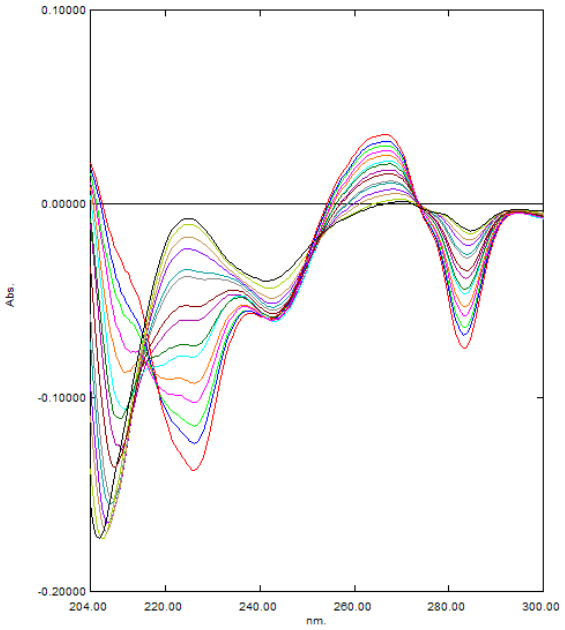

Figure 3: First derivative spectra of mixture contain (5-150 mg. L^{-1}) phenylephrine HCl in the presence of (100 mg. L^{-1}) amoxicillin

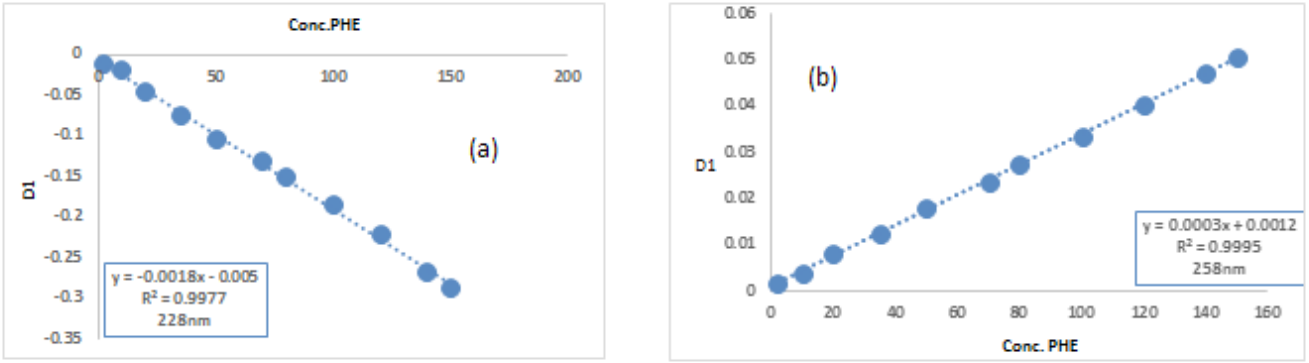

Figure 4: Calibration curves via first derivative spectra of phenylephrine HCl at (a) 228 nm, (b) 258 nm

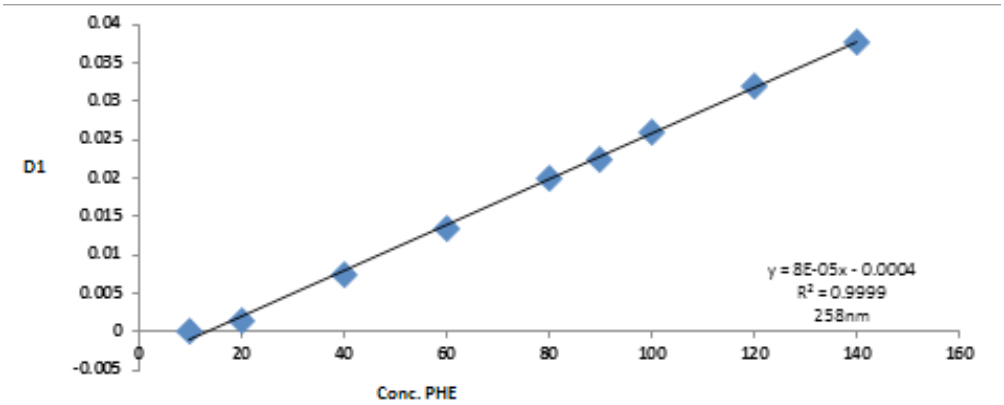

Figure 5: Calibration curves First derivative spectra of mixture contain (5-150 mg. L^{-1}) phenylephrine HCl in the presence of (20 mg. L^{-1}) amoxicillin at (A) 258 nm

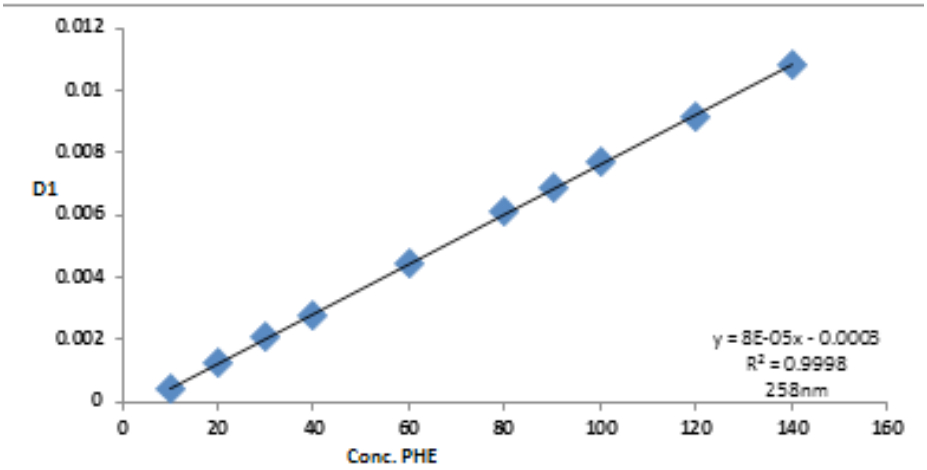

Figure (6): Calibration curves first derivative spectra of mixture contain (5-150 mg. L^{-1}) phenylephrine HCl in the presence of (100 mg. L^{-1}) amoxicillin at (A) 258 nm

Figures 1-3. The procedure showed good results over the studied range of concentration depending on zero cross measurements Figures 4-6 and show plotted calibration curves.

Simultaneous Calibration of Secondary Mixture via Derivative Mode

Secondary mixtures for the cited drugs with different ratios were prepared depending on the results obtained in the individual calibration so that to keep the upper absorption range of the prepared mixtures to be less than 3.0. The first step in the calibration is to select the more convenient analytical wavelengths at which the multi-component system is analyzed by derivative spectrophotometry.19 In practice, the selected wavelength exhibited the best linear response by giving a zero or nearby zero intercept with the ordinate of the calibration curve, and not affected by the presence of any other component.20

In zero-crossing technique, measurement of the derivative value at a wavelength, at which the derivative of the interfering species accepts value zero-crosses the zero line. This technique should be determined for at least two concentrations.21 In the present study, the zero-crossing method proved the utility for the simultaneous analysis of the cited drugs in their secondary mixtures. Calibration curves were constructed by plotting the value of derivative of the 1st and 2nd, order derivatives spectra against the corresponding concentrations of the examined drugs.22

The careful inspection of the second derivative spectra obtained for the mentioned mixtures of PHE shows, zero-cross measurements at a specified wavelength and could be used to quantify the exact concentration of PHE in the presence of AMX. Figures 7-8 show the calculated second derivative spectra of different mixtures of the cited drugs, while Figures 9-10 represent the calibration graphs.

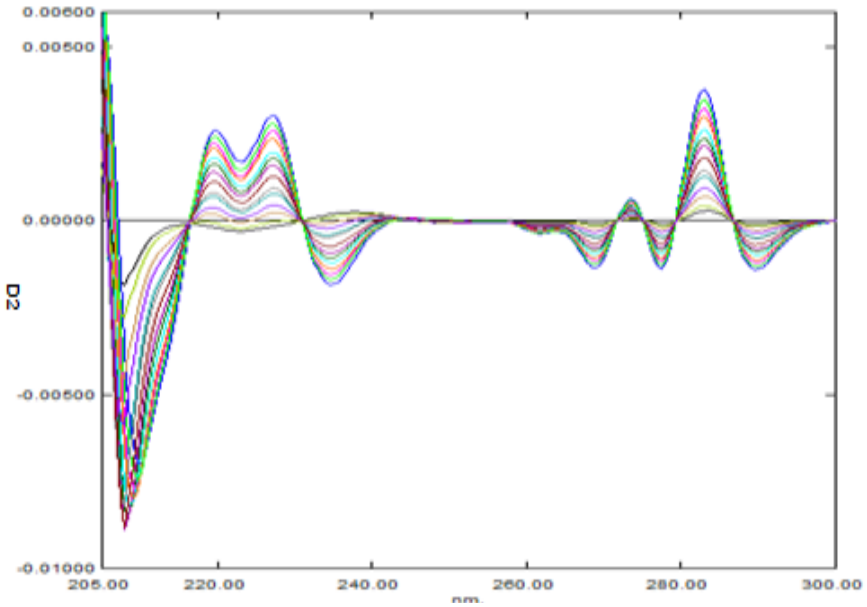

Figure 7: second derivative spectra of mixture contain (5-150 µg.mL^{-1}) phenylephrine HCl in the presence of (20 µg.mL^{-1}) amoxicillin

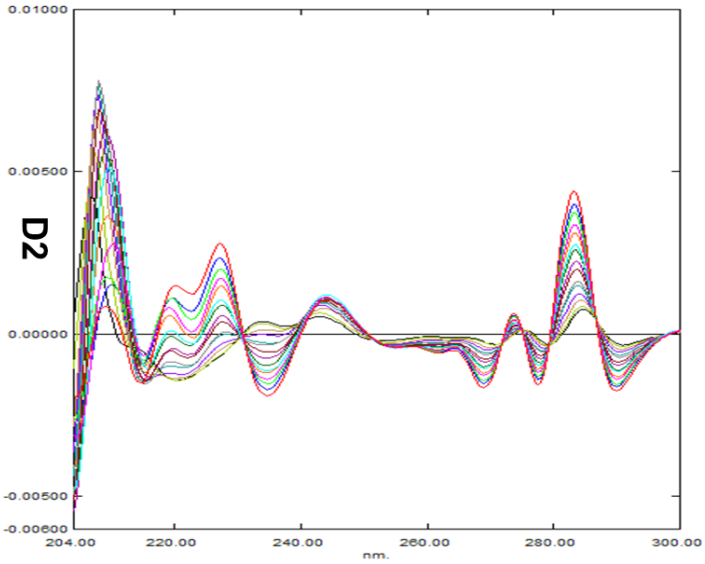

Figure 8: second derivative spectra of mixture contain (5-150 mg. L^{-1}) phenylephrine HCl in the presence of (mg. L^{-1}) amoxicillin

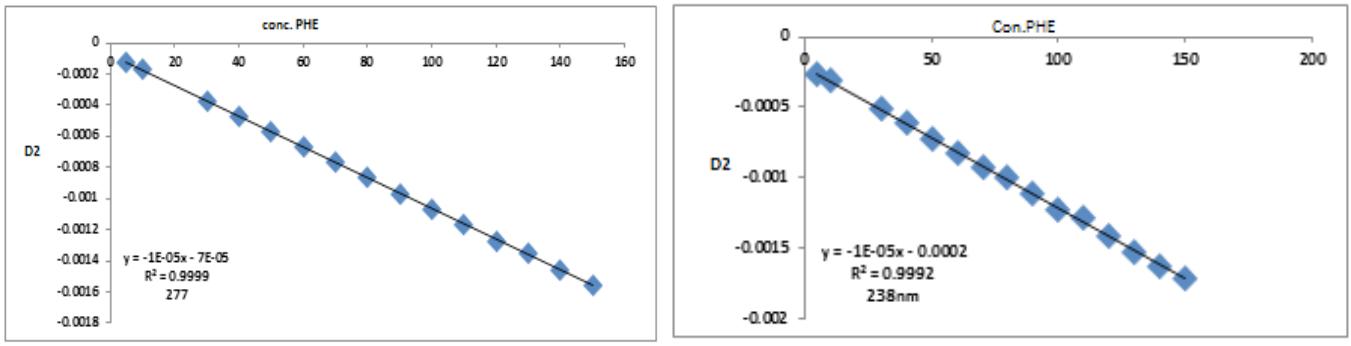

Figure 9: Calibration curves First derivative spectra of mixture contain (5-150 mg. L^{-1}) phenylephrine HCl in the presence of (20 mg. L^{-1}) amoxicillin at **(A)** 238 nm, **(B)** 277 nm

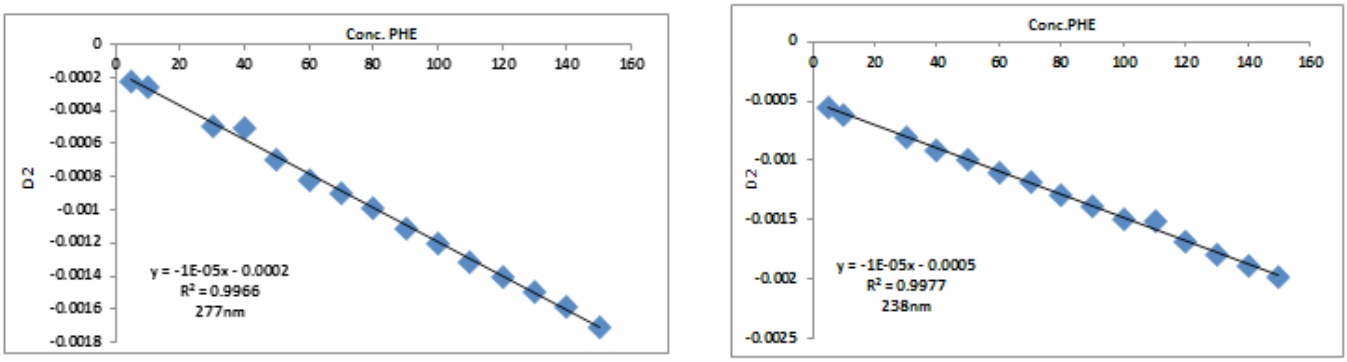

Figure (10): Calibration curves First derivative spectra of mixture contain (5-150 µg.mL^{-1} mg. L^{-1}) phenylephrine HCl in the presence of (100 mg. L^{-1}) amoxicillin at **(A)** 238 nm, **(B)** 277 nm

Calibration Curves for AMX

For the assay of AMX via UV-spectrophotometric method, Calibration curves were constructed operated under the mentioned optimum conditions for D1 and D2 modes in selected ranges of wavelengths. To utilize derivative modes for the quantitative analysis of PHE same ideas previously are used zero-cross measurements.

The recorded spectra using D1 mode for a set of solutions containing (2-100 mg.L^{-1}) AMX in the presence of different concentrations of PHE (0, 20,100 mg.L^{-1}) are shown in

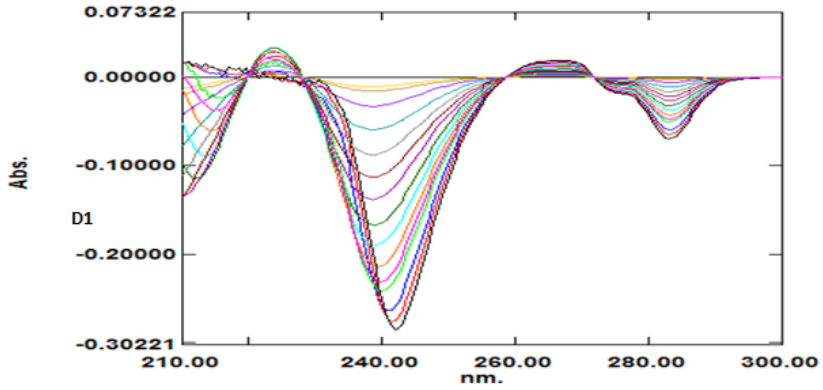

Figure 11: First derivative spectra of (5-240 mg.L^{-1}) Amoxicillin concentration.

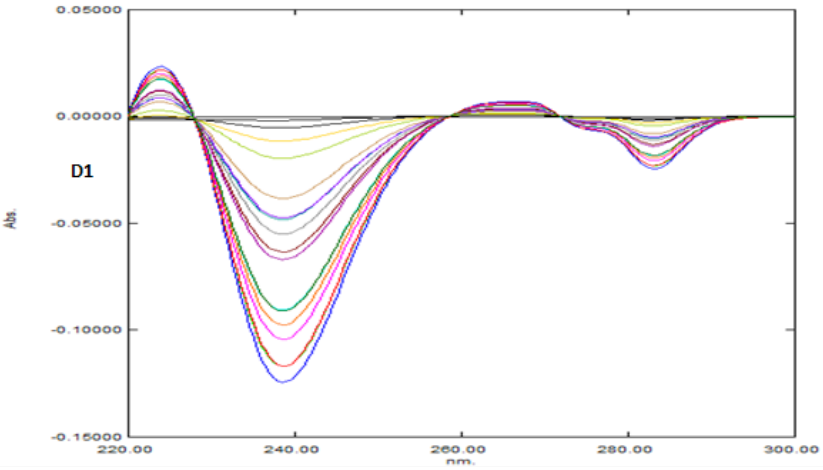

Figure 11(a): First derivative spectra of mixture contain (5-160 mg. L^{-1}) amoxicillin in the presence of (20 mg. L^{-1}) phenylephrine HCl

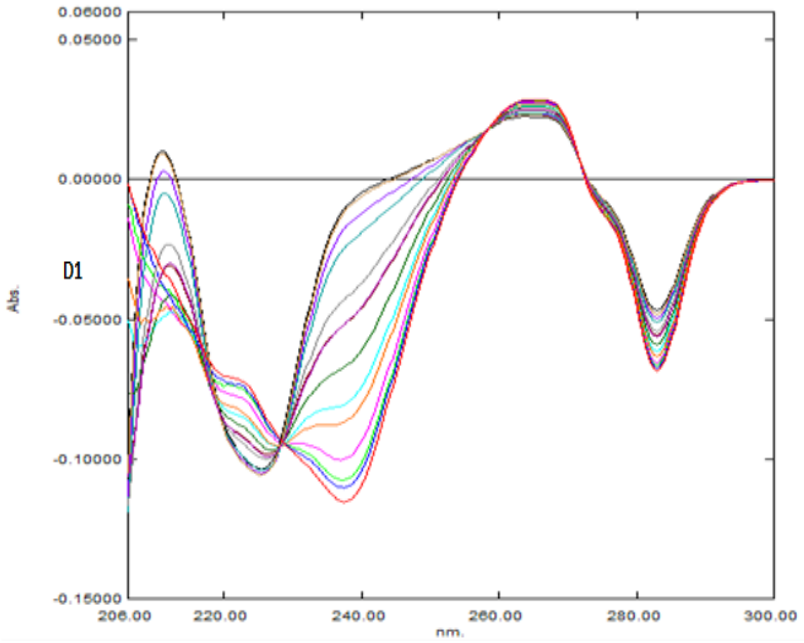

Figure 11(b): First derivative spectra of mixture contain (5-160 mg. L^{-1}) amoxicillin in the presence of (100 mg. L^{-1}) phenylephrine HCl.

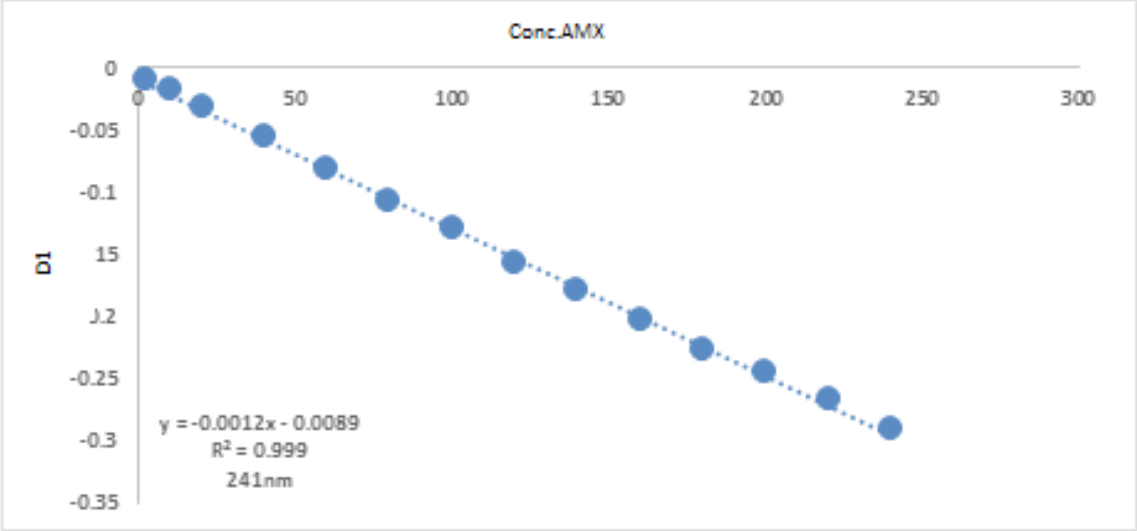

Figure 12: Calibration curves via the first derivative spectra of amoxicillin at 241nm

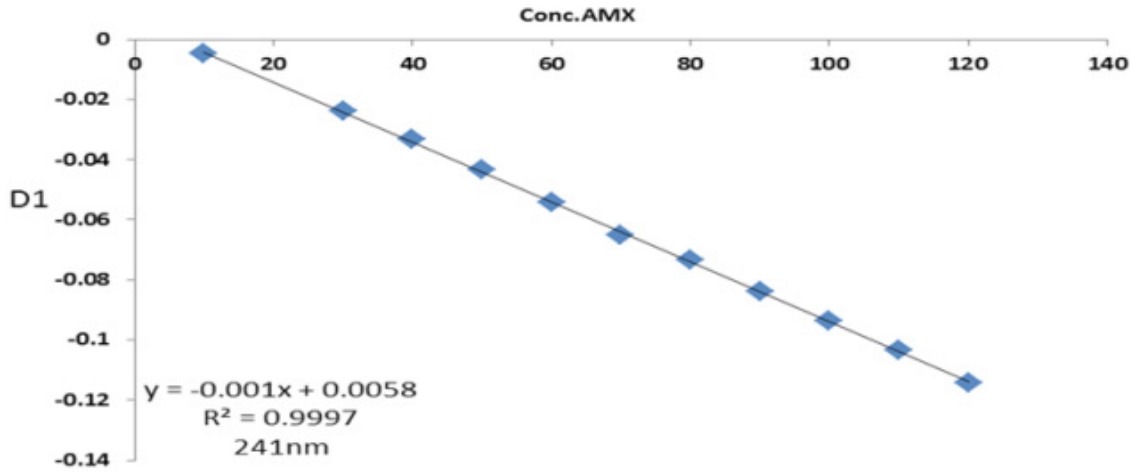

Figure 12a: Calibration curves First derivative spectra of mixture contain (5-150 mg. L^{-1}) amoxicillin in the presence of (20 mg. L^{-1}) phenylephrine HCl at 241 nm.

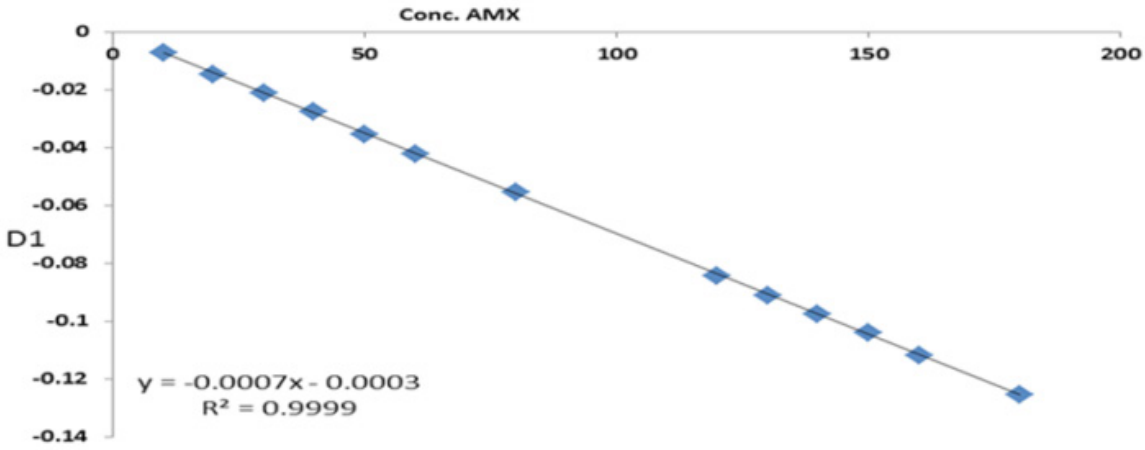

Figure 12(b): Calibration curves First derivative spectra of mixture contain (5-150 mg. L^{-1}) amoxicillin in the presence of (100 mg. L^{-1}) phenylephrine HCl at 241 nm.

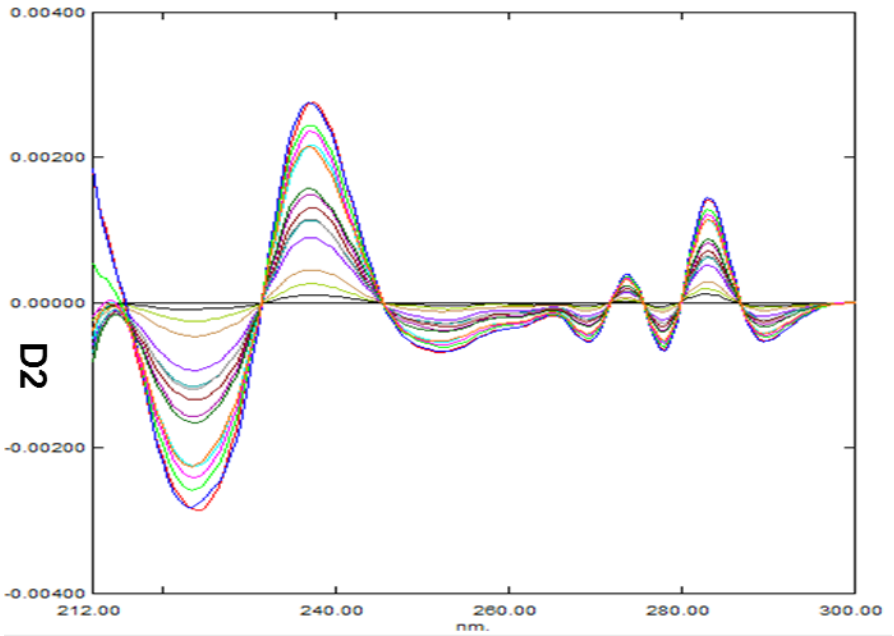

Figure 13: second derivative spectra of mixture contain (5-60 mg. L^{-1}) amoxicillin in the presence of (20 mg. L^{-1}) phenylephrine HCl.

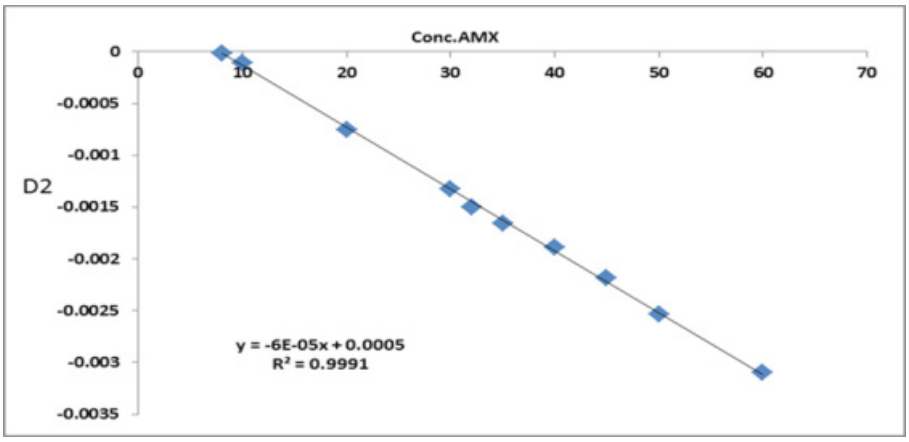

Figure 13(a): Calibration curves second derivative spectra of mixture contain (5-60 mg. L^{-1}) amoxicillin in the presence of (20 mg. L^{-1}) phenylephrine HCl at 226 nm.

Figures 4-6. The procedure showed acceptable results over the studied range of concentration depending on zero crosses, measurements Figures (7-9), and show plotted calibration curves.

Moreover, the second derivative spectra of the same sets of solution (i.e., containing (5-100 µg.mL-1) amoxicillin with different spiked concentrations of phenylephrine HCl (0, 20, 100 µg.mL^{-1}) were also recorded, and attempts were made to utilize them for finding the concentrations of the drug. Figures (4-20) - (4-24) show the D2 spectra for different concentrations of Amoxicillin and for its mixtures with phenylephrine HCl.

Selectivity

Selectivity of the methods was achieved by the analysis of different laboratory prepared mixtures of PHE and AMX within the linearity range, including the ratio present in the pharmaceutical dosage form. Satisfactory results were obtained, as shown in Table 1.

Stability

PHE and AMX working standard solutions in distilled water showed no spectrophotometric changes up to 2 weeks when stored at room temperature.

The linearity of the method was determined at concentration levels ranging from 2 to 150$mgL^{-1}$for phenylephrine HCl and 2 to180 $mgL^{-1}$for Amoxicillin The calibration curve was constructed by plotting response factor against the concentration of drugs the results show in Table 1.

Table 1: Summary of the selected methods for the determination of PHE , AMX, and analytical parameters.

Drug	*Taken range mg.L^{-1}*	*Derivative Mode*	*λ(nm)*	*Conc. mg.L^{-1}*	*Regression Equation*	*R^2*	*LOD mg.L^{-1}*	*LQD mg.L^{-1}*
PHE	5-150	D1 (zerocross)	258	20	y = 8*10-5x -0.0004	0.9999	0.16629	0.5543
		D2 (zero cross)	238		y = -1*10-5x -0.0002	0.9992	0.1643	0.5478
		D2 (zero cross)	277		y = -1*10-5x -7*10-5	0.9999	0.1239	0.4131
		D1 (zero cross)	258	100	y = 8*10-5x -0.0003	0.9998	0.0638	0.21277
		D2 (zerocross)	238		y = -1*10-5x -0.0005	0.9977	0.1528	0.5095
		D2 (zerocross)	277		y = -1*10-5x -0.0002	0.9966	0.1131	0.3771
AMX	5-180	D1 (zerocross)	241	20	y = -0.0007x - 0.0003	0.9999	0.1755	0.5850
		D2 (zerocross)	226		y = -6*10-5 x - 0.0005	0.9987	0.2304	0.7680
		D1 (zerocross)	241	100	y = -0.001x - 0.0058	0.9997	0.0332	0.11089

*Detection limit = 3.3 (SD / slope), n = 3 measurements.

Table 2: Evaluation of accuracy and precision for the determination of mixing AMX and PHE via derivative spectrophotometry.

Drugs	*Order of derivative*	*λ(nm)*	*conc.(mg. L-1)*		*E%*		
			Taken	**Found*		*Recovery*	*R.S.D%*
phenylephrine HCl	First	258	30	30.125	0.4166	100.416	0.02393
	second	238		31	3.3333	103.333	0.2290
		277		29	-3.3333	96.666	0.2448
	First	258	90	90.137	0.15277	100.152	0.00799
	second	238		89	-1.1111	98.8888	0.0655
		277		91	1.1111	101.111	0.06406
	First	258	120	118.625	-0.01146	98.8541	0.466
	second	238		119	-0.8333	99.1666	0.1557
		277		121	0.8333	100.833	0.15316
Amoxicillin	First	241	10	9.8714	-1.28571	98.714	0.1041
	second	226		10.11833	1.8333	101.833	0.10487
	First	241	30	30.0285	0.0952	100.095	0.05491
	second	226		30.3638	1.22778	101.2278	0.03516
	First	241	50	50.5	1	101	0.0571
	second	226		50.61	1.22	101.22	0.021103

Accuracy study

Recovery experiments determined the accuracy of the method. A study was performed by addition of different amounts of phenylephrine hydrochloride or amoxicillin to a known concentration of the pharmaceutical dosage forms. The percentage of recoveries were calculated.

CONCLUSION

Simple, rapid, and accurate method are described for the simultaneous determination of AMX and PHE in two-component mixtures. The comprised measurement of difference absorptivities derivatized in the first or second order. Absorbance ratio method was also developed for a comparison method with a high percentage of recovery, good accuracy and precision

PHE and AMX working standard solutions in distilled water showed no spectrophotometric changes up to 2 weeks when stored at room temperature.

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